Educational Administration: Theory and Practice
2024, 30(3), 2381-2388
ISSN: 2148-2403
https://kuey.net/

Research Article

In-silico evaluation of bioactive compounds from natural sources targeting NIK and NF-κB pathways for chronic prostatitis therapy and ADMET profile analysis

Vinayak Bhushan, Rakesh K. Sindhu*

School of Pharmacy, Sharda University, Greater Noida, India 201310, Email: drrakeshksindhu@gmail.com

Citation: Vinayak Bhushan, et al (2024), In-silico evaluation of bioactive compounds from natural sources targeting NIK and NF-κB pathways for chronic prostatitis therapy and ADMET profile analysis, *Educational Administration: Theory and Practice, 30(3), 2381-2388, Doi:* 10.53555/kuey.v30i3.5188

ARTICLE INFO

ABSTRACT

Inflammation, cancer and a variety of human disorders are connected with NF-KB and NF-κB -inducing kinase light-chain-enhancer of activated b cells increased expression in the biological framework. The NF-κB and NIK cascades vital components may control the relocation as well as stimulation of the NF-κB transcription factor along with NIK. The current work focuses on active site analysis as well as molecular docking investigations of NF-κB and NIK pathway key components. The natural chemicals employed as ligands in this docking investigation are Guggulsterone Z and Withaferin A. The present study focused on comparative docking studies on drug target proteins using separate ligands. The current study shed information on the binding capacities and interactions of components found in the oleoresin of Ashwagandha and Guggul plants with the active sites of NFkB and NIK. The molecular docking studies predicted an improved interaction between NFkB NIK, and the bioactive content of Ashwagandha (Withaferin A) and Guggul (Guggulsterone Z (GZ)) with binding energies of -7.5, -8.8, -7.1, and -8.2 kcal/mol, confirming the coexistence of both ligands at different binding sites of the metabolising enzymes. Furthermore, the ADMET and drug-likeness ratings were shown to be beneath the normal range for water solubility, Caco-2 permeation, human intestinal absorption (HIA), and blood-brain barrier (Absorption) and exhibited negative cancer-causing consequences.

Keywords: Phytoconstituents, docking study, NIK, NFkB, chronic prostatitis

Introduction

Ayurveda, frequently acknowledged as “The Science of Life”, is an ancient form of medicine practiced in the Indian subcontinent. It is centred on an all-encompassing approach to life, health, including healing. Ayurvedic texts explain rejuvenate treatments that replenish the body's tissues. This study uses medicinal herbs such as Withania somnifera (Ashwagandha) and Commiphora wightii (Guggul) that have therapeutic potential for respiratory ailments and many other ailments. They both act as immunomodulators, strengthening the body's defences against infections[1]. W. somnifera (WS), also known as “Ashwagandha”, is a “rasayana” (rejuvenator) that improves physical along with mental well-being, rejuvenates the body, and promotes longevity. It has been shown to have the qualities include anti-inflammatory, anti-hyperglycaemic, anti-microbial, analgesic, anti-tumor, de-stress, neurologically protective, cardicprotection, revitalising & immunobalancing. Guggul's traditions dates back to 1700 BC in line with Sushrut Samhita, a medical and surgical text, oral administration of guggul can treat internal tumours, malignant ulcers, obesity, liver dysfunction, intestinal parasites, leucoderma vulgar, sinusitis, as well as edema. This conventional form of medicine serves to prevent & treat several conditions, including IBD, ulcerations, arthritis, CVDs, in addition to hyperglycemia[2,9].In carcinogenesis, signalling pathways are less well regulated. Signalling pathway dysregulation is a major contributor to cancer and inflammatory disorders. The NF-κB signalling system is a complicated network that connects extracellular stimuli with cell survival and proliferation. It responds to several activators, including“viruses, the cytokines interleukin, lipopolysaccharides (LPS), oxygen depletion, along with substances that negatively impact DNA”[3]. NF-κB (nuclear factor kappa) can activate either conventional or alternative signalling pathways. Different cues excite these pathways, which need distinct IκB kinase (IKK) complexes. Many receptors preferentially activate the alternative route. The classical signalling pathway has

received significant attention as a therapeutic target. There is a rising interest in creating powerful modulators to block NF-κB function by inhibiting different phases of the signalling cascade [4]. To reduce growth of cancerous tumour & medication resistance, it is vital to decrease upstream proteins that activate NF-kB. NF-kB -inducing kinase NIK is a critical gatekeeper kinase that regulates stimulation of the NF-kB route plays a considerable part in modulating immunity as well as inflammation furthermore because it affects vital cellular processes that encompass proliferation division as well as cell viability NIK constitutes a prerequisite for the optimum operations of the cells[5].Molecule docking is an executable programme known to examines the search zone indicated by the molecular proximity and ranks options to determine the optimum adherence behaviour. Thus, docking needs both a search technique and an assessment mechanism.Docking relies of a pair of primary steps: sampling along with scoring. Sampling implies an exhaustive examination of the conformational domain of the substances that are getting docked. This dynamic area is enormous, attributable partially to the flexibility of both the receptor as well as the ligand, which allows every molecular structure to change shape in response to the other's effect. To restrict this wide conformational area, the receptor is usually maintained stiff[6].

2. Methodology

2.1 Protein Preparation

The crystalline structures of the NIK Complex "PDB ID: 4G3D" and NF-κB "PDB ID: 3GUT" were acquired from the Protein Data Bank (http://www.rcsb.org/)", at the beginning of study all non-protein molecules were removed from the docking simulations, and just the required atom site was kept for any alternative atom positions. All target proteins were energy-minimized prior to docking studies[7].

2.2 Ligand Preparation

The 3D structures of Guggulsterone Z ""(8R,9S,10R,13S,14S,17Z)-17-ethylidene-10,13-dimethyl-1,2,6,7,8,9,11,12,14,15-decahydrocyclopenta[a]phenanthrene-3,16-dione)(mw: 312.4 g/mol))" and Withaferin A "((1S,2R,6S,7R,9R,11S,12S,15R,16S)-6-hydroxy-15-[(1S)-1-[(2R)-5-(hydroxymethyl)-4-methyl-6-oxo-2,3-dihydropyran-2-yl]ethyl]-2,16-dimethyl-8-oxapentacyclo [9.7.0.02,7.07,9.012,16] octadec-4-en-3-one) (mw: 470.6 g/mol)" " were retrieved from the Pubchem database "(http://pubchem.ncbi.nlm.nih.gov/)" MMFF94x force feld was used to optimise the results (Figure 1(a)(b))". The optimised structures were utilised as starting points for molecular docking investigations.

(Z)-Guggulsterone **Withaferin A**

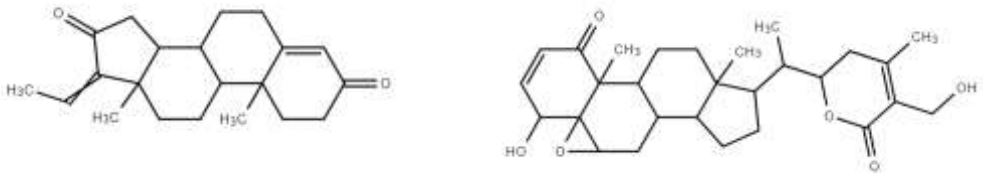

Figure 1 (a)(b) - Structure of Guggulsterone Z and Withaferin A

2.3 Molecular docking

The molecular docking investigation was conducted using AutoDock Vina software. YASARA was used to dock active phytochemicals from W. somnifera (Withaferin A) and Commiphora wightii (Guggulsterone Z) with NFkB Mpro (PDB ID: 1NFK) and NIK (PDB ID: 4G3D). To conduct the docking investigation, the study utilised prepared receptor and ligand files to define targets as well as play macros in Autodock Vina programme. The macro file dockrun_mcr was used to calculate the interaction energy between the receptor along with chosen ligands individually. YASARA was used to perform 25 VINA docking sessions between ligand 2 and receptor 1. Docked complexes were visualised using YASARA software and exported to PDB files for interactive 2D-3D visualisation using DS 4.0 and PyMol. The docking calculation investigation used result log data from Autodock Vina.

Docked complexes were reduced in size using Vina scoring to calculate binding energy (kcal/mol) and dissociation constant (pM). Positive energy suggests superior binding, whereas energy that is negative indicates no possible binding[4].

2.4 Molecular dynamic stimulation

The docked complexes were prepared, maximised, along with minimised using the protein synthesis tool in the"OPLS-3e (optimised potential for liquid simulations-Schrodinger)" force field. "Molecular dynamics (MD)" simulations using the Desmond MD software were performed on complexes with the least energy and RMSD of 0.30 Å. The system builder was used to mask protein-ligand complexes by executing the TIP3P water model. To neutralise, metal ions were supplied at a salt concentration of 0.15. The built-in systems in each complex were subjected to a typical equilibration technique. MD simulation was done at a timeframe of 20 ns using the "OPLS-3e" force field. The Desmond simulation interaction analysis was used to investigate RMSD and hydrogen bonding[4,8-10].

2.5 Drug Likeness and ADMET prediction

Using the web-based Lipinski rule of five (http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp) along with admetSAR server (http://lmmd.ecust.edu.cn/admetsar1/predict/), the most promising docked compounds from Ashwagandha (Withaferin A) as well as Guggul (Guggulsterone Z) were selected for druglikeness testing as well as "ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) profile prediction" [6, 11].

The Secondary metabolic profiling, antioxidant potential, enzyme approach estimates the penetration of tiny chemical substances by estimating their lipophilicity along with polarity, making it an accurate predictor. The model predicts brain as well as intestine penetration based on the same physicochemical characteristics, making it easy to convert into molecular design due to its quickness, precision, conceptual simplicity, and clear graphical output[12].

3. Results

3.1 Molecular docking

Different active phytochemicals found in Ashwagandha (Withaferin A) & Guggul (Guggulsterone Z) had a considerable binding affinity with NFkB as well as NIK, according to a molecular docking research that assessed YASARA score in order to exhibit significant binding affinity with of NIK and NFkB. In accordance with molecular docking investigations, Withaferin A and Guggulsterone Z have a strong binding affinity. Withaferin A, when combined with NIK and NFkB, has a maximal binding energy of -7.5 and -8.8 kcal/mol. When combined with NIK and NFkB, Guggulsterone Z had a highest binding energy of -7.1 and -8.2 kcal/mol as shown in image. There were few van der Waals contacts created by residual residues. The standard binding range for considerable binding is (- 3.2 and -18.5 kcal/mol)[10].

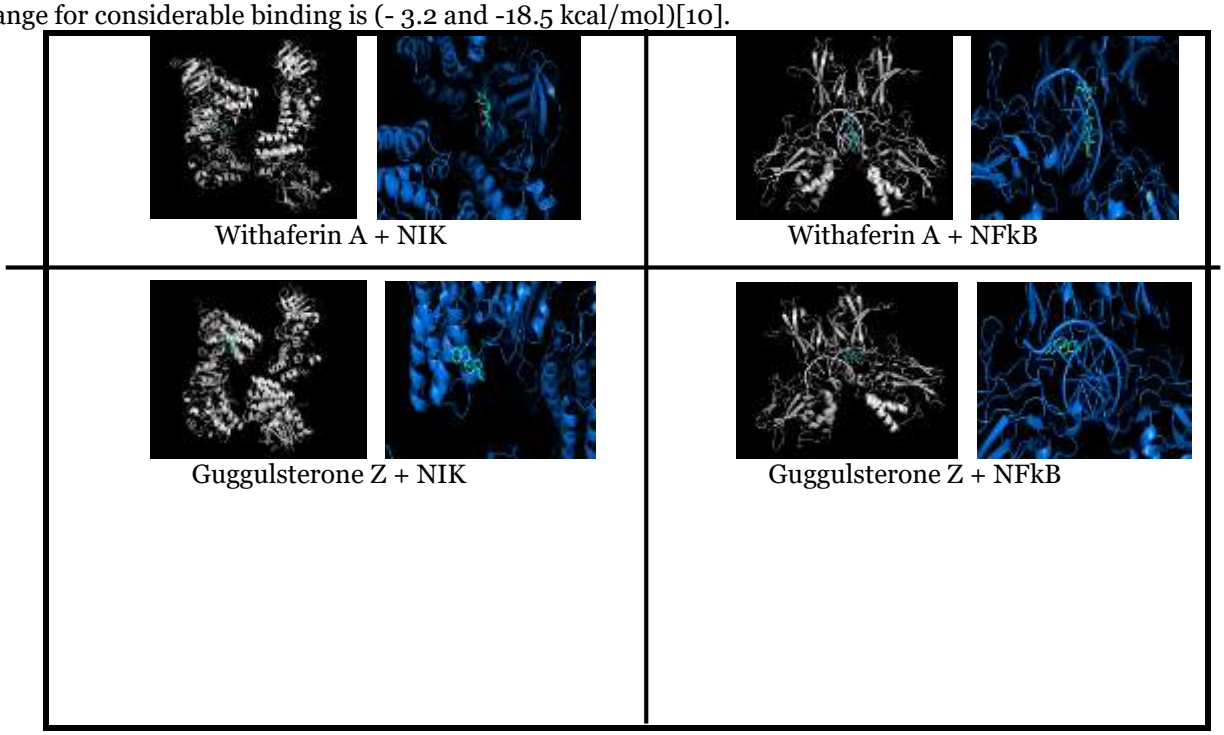

Figure 2- Docked structures of Withaferin A and Guggulsterone Z with NIK and NFkB (a)(b)(c)(d) 1-Withaferin A +NIK, Withaferin A + NFkB, Guggulsterone Z +NIK, Guggulsterone Z +NFkB

The group of phytochemicals having considerable binding energy (- 3.2 and -18.5 kcal/mol kcal/mol) with NFkB along with NIK is shown in Table 1.

Table 1. List of phytochemicals with binding energy - 3.2 and -18.5 kcal/mol for NFkB and **NIK**

Compounds	Target molecule; Binding energy (kcal/mol) (- 3.2 and -18.5 kcal/mol) (5)
Withaferin A + NIK	-7.5
Withaferin A + NFkB	-8.8
Guggulsterone Z + NIK	-7.1
Guggulsterone Z + NFkB	-8.2

3.2 Molecular dynamics simulations

The covariance matrix reveals the interaction between pairs of residues, which means whether these residues undergo movements could be linked (red), independently occurring (white), or inversely associated (blue). By IMODS ligand and protein prep by MDWEB (Molecular dynamics on web).

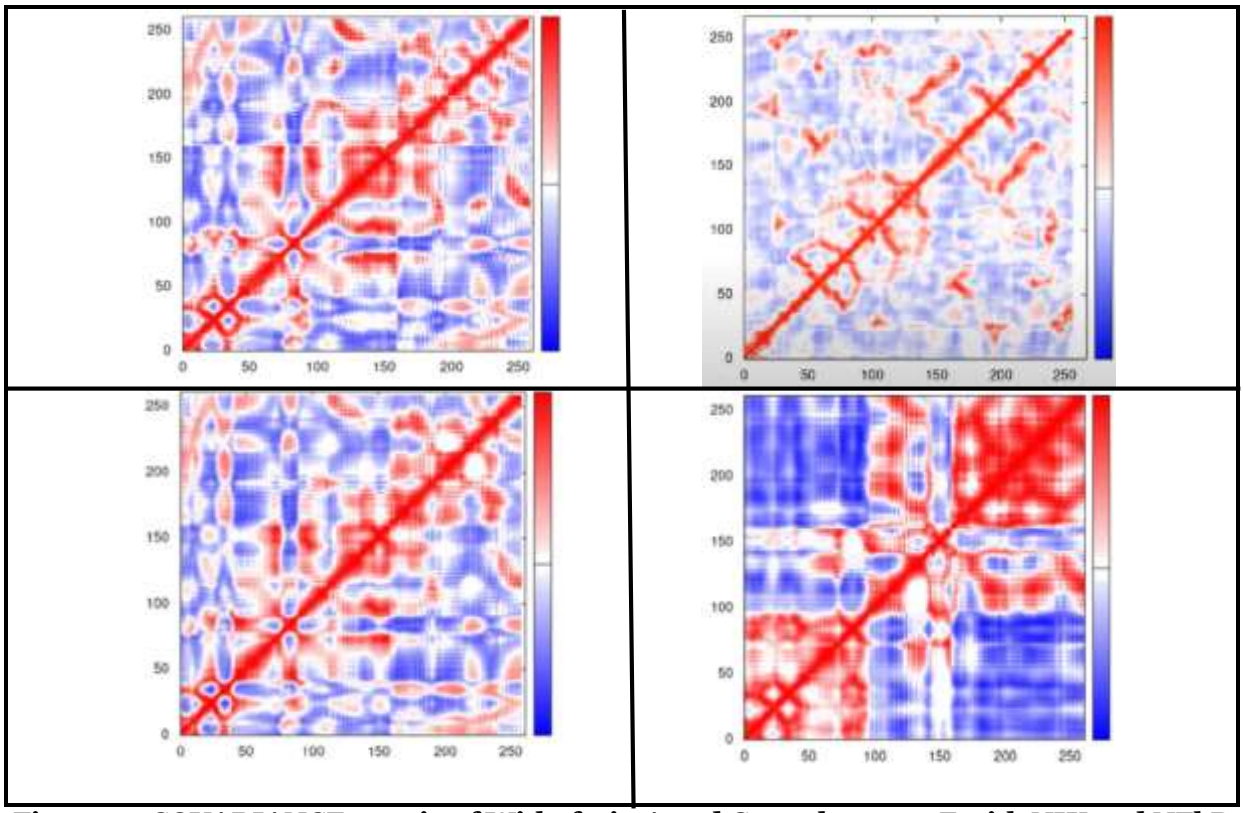

Figure 3 - COVARIANCE matrix of Withaferin A and Guggulsterone Z with NIK and NFkB (a)(b)(c)(d) 1-Withaferin A + NIK, Withaferin A + NFkB, Guggulsterone Z + NIK, Guggulsterone Z + NFkB

3.3Drug-likeness and ADMET profile analysis

The ADMET molecular prediction of property test was executed up via the swissadme server, and the drug-likeness test for the best docked compounds was determined by applying Lipinski's rule of five[11,13]. By adhering to its five parameters—molar refractivity, log P, hydrogen bond donor, hydrogen bond acceptor, as well as molecular mass—the Lipinski rule of five, referred to as the thumb rule of five, helps distinguish among compounds that are considered drug-like and those that are not. In terms of their water solubility, permeability to Caco-2, human intestinal absorption (HIA), and blood-brain barrier (Absorption). Withaferin A and Guggulsterone Z were determined to fall within the usual range and to have no carcinogenic (NC) effects (Table 2)[13].

Table 2. Physiochemical properties of potential inhibitors of NFkB and NIK receptor from Withaferin A and Guggulsterone Z

Compound name	MW < 500	Blood-Brain Barrier (Absorption)	Aqueous solubility	HIA	Caco-2 (cm/s)	Carcinogens
Withaferin A	470.6g/mol	0.8697	-4.2028	1	0.6967	NC
Guggulsterone Z	312.4g/mol	0.982	-4.3674	0.8086	0.7724	NC

- **For Guggulsterone Z**

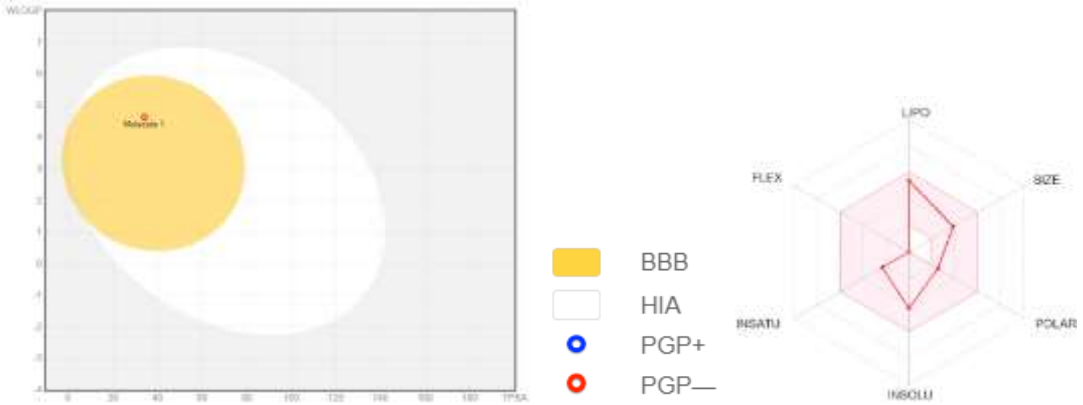

Figure 4 - A BOILED-Egg illustration to predict gastrointestinal absorption and brain penetration of small molecules.

P-gp substrates (PGP+), P-gp non substrates (PGP-), Human gastrointestinal absorption (HIA)

Log S (SILICOS-IT) - 4.58 mol/l	**Solubility** - 8.14e-03 mg/ml; 2.61e-05 mol/l **Class** - Moderately soluble

Pharmacokinetics					
GI absorption	High	**BBB permeant**	Yes	**P-gp substrate**	No
CYP1A2 inhibitor	No	**CYP2C19 inhibitor**	Yes	**CYP2C9 inhibitor**	Yes
CYP2D6 inhibitor	No	**CYP3A4 inhibitor**	No	**Log (Skin Permeation)**	5.41 cm/s

- **For Withaferin A**

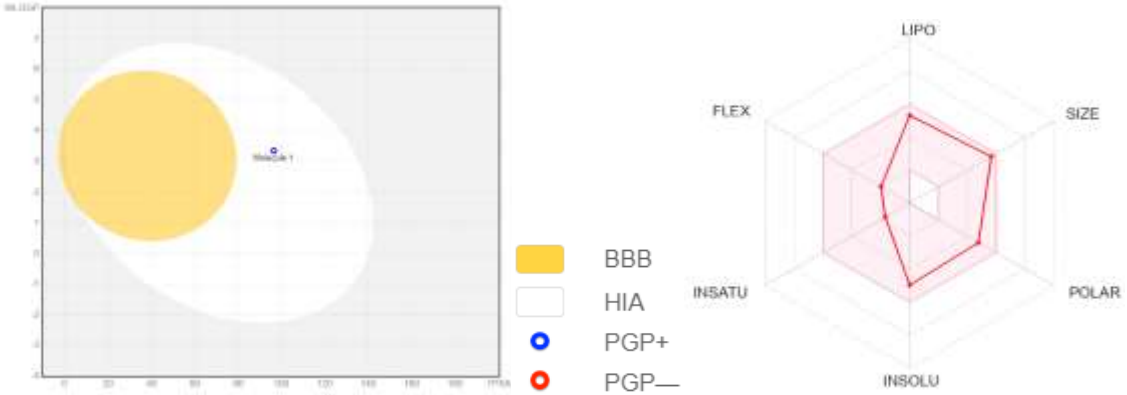

Figure 5 - A BOILED-Egg illustration to predict gastrointestinal absorption and brain penetration of small molecules.

P-gp substrates (PGP+), P-gp non substrates (PGP-), Human gastrointestinal absorption (HIA)

Lipophilicity			
Log Po/w (iLOGP) -	3.39	**Log Po/w (MLOGP) -**	2.75
Log Po/w (WLOGP) -	3.35	**Log Po/w (XLOGP3) -**	3.83
Consensus Log Po/w -	4.03	**Log Po/w (SILICOS-IT) -**	4.74

Water Solubility			
Log S (ESOL) -	4.97 mol/l	**Solubility -**	5.01e-03 mg/ml; 1.07e-05 mol/l
		Class -	Moderately soluble
Log S (Ali) -	5.55 mol/l	**Solubility -**	1.37e-02 mg/ml; 4.40e-05 mol/l
		Class -	Moderately soluble
Log S (SILICOS-IT) -	3.79 mol/l	**Solubility -**	7.54e-02 mg/ml; 1.60e-04 mol/l
		Class -	Moderately soluble

Pharmacokinetics					
GI absorption -	High	**BBB permeant -**	No	**P-gp substrate -**	Yes
CYP1A2 inhibitor -	No	**CYP2C19 inhibitor -**	No	**CYP2C9 inhibitor -**	Yes
CYP2D6 inhibitor -	No	**CYP3A4 inhibitor -**	No	**Log(SkinPermeation)- -**	6.45 cm/s

4. Discussion

In contemporary medicine, anti-inflammatory and corticosteroid medication are being utilised singly or in combination to treat chronic prostatitis. Since natural products have a lower toxicity profile than manufactured substances, they can be employed medicinally. When seeking treatment for chronic prostatitis, turning to Ayurveda offers dependable, scientifically supported herbal remedies to address respiratory ailments and many other ailments like inflammation, diabetes, microbial infection, analgesic, tumor, stress, neurological disease, chronic heart disease. The present treatment approach is thought to benefit from the addition of techniques to decrease NF-κB activity in malignancies. In this work, study conducted the first docking investigation of Guggulsterone Z and Withaferin A on NF-κB signalling proteins [14-16].The IKK complex is a crucial part of the NF-κB signalling cascade, which phosphorylates Ser32 and Ser36 to activate IκBα. This is accomplished by activating the NIK complex [9,18-20].NIK overexpression causes NF-κB hyperactivation in cancer cells. Catalytic IKKα/β kinases and a regulatory protein (NEMO, or IKKγ-NFkB essential modulator) make up the NIK related IKK complex. When NEMO binds to the unphosphorylated IKK kinase C termini, the IKK complex's catalytic activity is triggered. The amino acid residues from NEMO (Leu93, Phe97, Lys90, Glu89, and Val104) and IKK kinase (W741, W739, and F734) contributed to the protein–protein interactions of additional IKK and NEMO [21]. In this investigation, the study found that by interacting with the active site, Guggulsterone Z and Withaferin A both interfered with the connection between NIK and the NF-κB complex. The active site residues that make up the NF-κB complex's DNA binding pocket primarily interact with DNA during NF-kB-dependent gene expression. Reduced binding interactions with DNA may result in the suppression of gene expression that is dependent on NF-kB. In acute prostate inflammation, pathways such as PI3K/Akt, MAPK, and NFB signalling are suppressed by Withaferin A and Guggulsterone Z. Numerous studies have shown that Guggulsterone Z is a highly effective NF-κB inhibitor in a range of malignancies and inflammatory illnesses. It was not previously known how Withaferin A and Guggulsterone Z together affected NF-κB or NIK inhibition. In this work, NIK and NF-κB are used for bioinformatics investigation of the binding processes of Withaferin A and Guggulsterone Z.

5. Conclusions

The term chronic prostatitis is ambiguous and describes a range of symptomologies that necessitate customised care based on the complaints of the patient. Given this, the study aim was to evaluate the strength of the evidence supporting various treatment alternatives and provide a treatment algorithm based on the widely discussed up point framework. The study found that there is possible association between Guggulsterone Z and Withaferin A on NFkB and NIK pathway which opens a wide range of opportunities for treatment of various indication which have chronic inflammation of prostate gland, which will ultimately increase human survival.

References

1. Shishodia S, Harikumar KB, Dass S, Ramawat KG, Aggarwal BB. The guggul for chronic diseases: ancient medicine, modern targets. Anticancer Res. 2008;28(6A):3647–3664.
2. Kunnumakkara, A. B., Banik, K., Bordoloi, D., Harsha, C., Sailo, B. L., Padmavathi, G., Roy, N. K., Gupta, S. C., & Aggarwal, B. B. (2018). Googling the Guggul (Commiphora and Boswellia) for Prevention of Chronic Diseases. Frontiers in pharmacology, 9, 686. https://doi.org/10.3389/fphar.2018.00686
3. Baeuerle, P. A., & Baltimore, D. (1996). NF-kappa B: ten years after. Cell, 87(1), 13–20. https://doi.org/10.1016/s0092-8674(00)81318-5
4. Thu, Y.M. et al. (2011) 'NF-KB inducing kinase (NIK) modulates melanoma tumorigenesis by regulating expression of pro-survival factors through the β-catenin pathway', Oncogene, 31(20), pp. 2580–2592. doi:10.1038/onc.2011.427.
5. Cheemanapalli, S., Chinthakunta, N., Shaikh, N.M. et al. Comparative binding studies of curcumin and tangeretin on up-stream elements of NF-κB cascade: a combined molecular docking approach. New Model Anal Health Inform Bioinforma 8, 15 (2019). https://doi.org/10.1007/s13721-019-0196-2
6. Shree, P., Mishra, P., Selvaraj, C., Singh, S. K., Chaube, R., Garg, N., & Tripathi, Y. B. (2020). Targeting COVID-19 (SARS-CoV-2) main protease through active phytochemicals of ayurvedic medicinal plants – Withania somnifera (Ashwagandha), Tinospora cordifolia (Giloy) and Ocimum sanctum (Tulsi) – a molecular docking study. Journal of Biomolecular Structure and Dynamics, 1–14. https://doi.org/10.1080/07391102.2020.1810778
7. H.M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, I.N. Shindyalov, P.E. Bourne, The Protein Data Bank (2000) Nucleic Acids Research 28: 235-242 https://doi.org/10.1093/nar/28.1.235.
8. McNutt, A.T., Francoeur, P., Aggarwal, R. et al. GNINA 1.0: molecular docking with deep learning. J Cheminform 13, 43 (2021). https://doi.org/10.1186/s13321-021-00522-2
9. Tripathi, M. K., Singh, P., Sharma, S., Singh, T. P., Ethayathulla, A. S., & Kaur, P. (2020). Identification of bioactive molecule from Withania somnifera (Ashwagandha) as SARS-CoV-2 main protease inhibitor. Journal of Biomolecular Structure and Dynamics, 1–14. https://doi.org/10.1080/07391102.2020.1790425
10. Elijah, S., Uba, S., & Adamu Uzairu. (2019). Molecular docking study for evaluating the binding mode and interaction of 2, 4-disubstituted quiloline and its derivatives as potent anti-tubercular agents against Lipoate protein B (LipB). Turkish Computational and Theoretical Chemistry/Turkish Computational and Theoretical Chemistry :, 3(1), 17–24. doi.org/10.33435/tcandtc.458615
11. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci. Rep. (2017) 7:42717.
12. iLOGP: a simple, robust, and efficient description of n-octanol/water partition coefficient for drug design using the GB/SA approach. J. Chem. Inf. Model. (2014) 54(12):3284-3301.
13. A BOILED-Egg to predict gastrointestinal absorption and brain penetration of small molecules. ChemMedChem (2016) 11(11):1117-1121.
14. Muhammad Imran Tousif, Abbas, Z., Nazir, M., Saleem, M., Saba Tauseef, Hassan, A., Ali, S., Ahmed, M., Khan, J., Gokhan Zengin, Hashem, A., Almutairi, K. F., Graciela Dolores Avila-Quezada, & Elsayed Fathi Abd-Allah. (2024). Secondary metabolic profiling, antioxidant potential, enzyme inhibitory activities and in silico and ADME studies: a multifunctional approach to reveal medicinal and industrial potential of Tanacetum falconeri. BMC Complementary Medicine and Therapies, 24(1). https://doi.org/10.1186/s12906-024-04459-5
15. Cheemanapalli, S. et al. (2019) 'Comparative binding studies of curcumin and Tangeretin on up-stream elements of NF-KB CASCADE: A combined molecular docking approach', Network Modeling Analysis in Health Informatics and Bioinformatics, 8(1). doi:10.1007/s13721-019-0196-2.
16. Zhou, L. et al. (2014) 'Disulfide-mediated stabilization of the IKB kinase binding domain of NF-KB Essential Modulator (NEMO)', Biochemistry, 53(50), pp. 7929–7944. doi:10.1021/bi500920n.
17. L Lipinski, C. A. (2004). Lead- and drug-like compounds: the rule-of-five revolution. Drug Discovery Today: Technologies, 1(4), 337–341. doi.org/10.1016/j.ddtec.2004.11.007
18. Jayaram, B. et al. (2012) 'Sanjeevini: A freely accessible web-server for target directed lead Molecule Discovery', BMC Bioinformatics, 13(S17). doi:10.1186/1471-2105-13-s17-s7.

19. Kielian T. (2009). Overview of toll-like receptors in the CNS. Current topics in microbiology and immunology, 336, 1–14. https://doi.org/10.1007/978-3-642-00549-7_1
20. Cheng, F., Li, W., Zhou, Y., Shen, J., Wu, Z., Liu, G., Lee, P. W., & Tang, Y. (2012). admetSAR: a comprehensive source and free tool for assessment of chemical ADMET properties. Journal of chemical information and modeling, 52(11), 3099–3105. https://doi.org/10.1021/ci300367a
21. Thapa, S., Biradar, M. S., Nargund, S. L., Ahmad, I., Agrawal, M., Patel, H., & Lamsal, A. (2024). Synthesis, Molecular Docking, Molecular Dynamic Simulation Studies, and Antitubercular Activity Evaluation of Substituted Benzimidazole Derivatives. Advances in pharmacological and pharmaceutical sciences, 2024, 9986613. https://doi.org/10.1155/2024/9986613